MAINE

Department of Human Services
Health & Environmental
Testing Laboratory

HETL

SUMMER **NEWSLETTER** 1999

Did We Do Something ?? WRONG ??

Could We Do Something ?? BETTER ??

See article on page 2!!!

MORBIDITY REPORT

Disease Period Covered: 1/1/99 to 6/26/99

MEASLES (RUBELLA)	0
MUMPS	0
RUBELLA	0
CRS (Congenital Rubella Syndrome)	0
DIPHTHERIA	0
TETANUS	0
PERTUSSIS	9
HIB	0

Data derived from the Maine Bureau of Health Immunization Program, Weekly Morbidity of confirmed cases.

WHAT YOU WILL FIND IN THIS ISSUE:

The Day Care Project has Begun.

by: Michael Corbin

Over the last year, the HETL, DEP, Maine Childhood Lead Poisoning Prevention Program (MCLPPP), and Day Care Licensing has been working on the details of lead inspections for licensed day cares. The project is now under way. The day cares are first inspected by the Day Care Licensing group. The inspectors are using a form developed to rate the day cares on potential lead hazards. If they receive a score of 6 or higher, a private lead inspector is sent to evaluate the situation and take paint, soil, dust wipe, and water samples. There are approximately 4,000 day cares to be inspected over the next year and so far 40 day cares have potential lead hazards.

Another project in the works is with the Maine State Housing Authority's HUD grant for the removal of lead from low income housing. This project will be starting this summer, but for now it is still in the organizational development stage. Stay tuned for more information to come.

The MCLPPP Advisory Committee and The Barbara Bush Children's Hospital have been working on a lead screening survey for the registered physicians within the state about how they screen children for lead poisoning potential. They are still collecting data from this survey and will have final results by the end of the summer.

The MCLPPP Advisory Education Sub-Committee and the Maine State Housing Authority are currently working together on a media campaign for the HUD grant and general lead screening. The first public service announcement may have a famous Maine celebrity. Watch for the PSA's. We currently have worked a deal with Stan Bennett, owner of Oakhurst Dairy, to place lead information on the milk cartons he produces. The information will be on the pint size all the way up to the gallon size. Oakhurst Dairy serves all of Maine and parts of New Hampshire and Massachusetts.

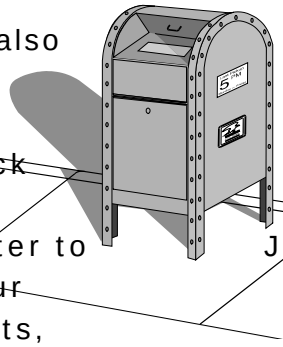
The Environmental and Blood Lead sections have created an information guide for lead analysis and are currently working on updating the Lab's web page.

Quality Assurance

by: Richard French, QAO

Complain, **complain**, **and complain** again II. Yes, again we at the HETL are asking for your help. In June the HETL will be including with all water test kits a questionnaire on the services we provide. Please take the time to fill it out and return it with your samples. The surveys will be compiled and used again to improve our service to you. We appreciate your response in the last survey. A number of changes were made which improved service, because of your feedback. If you would like to contact me by e-mail, my address is **richard.french@state.me.us**. Our fax number is **287-6832**. We welcome your response.

Guess What!



You can also use the mail card on the back of this newsletter to send your comments, suggestions, or problems back to us. It's as easy as 1-2-3! Just compose, cut, and mail.

Drinking Water Inorganics and Microbiology

by: Richard French , Supervisor

The month of May is the beginning of our seasonal increase in samples, of due to the opening the summer establishments for the influx of seasonal tourists. We at HETL and Health Engineering have received an increased number of calls on the kits that have been sent out and the necessity of testing. To clear up some of the confusion, I will restate the information on testing that was in the Fall newsletter. The sample kits that are now sent to water supplies consists of two kits, one marked TG1 for bacteria and the other marked NO3N for nitrate nitrogen. You may also be sent a TG1 monthly for the months you are open. It is very important that you send in all of the compliance samples sent to you. About 99% of the time the testing is necessary for compliance. If your bacteria sample is "positive" for coliforms, you will be required to take four more bacteria recheck samples and may be required to take five more bacteria samples the following month as part of the EPA Total Coliform Rule. If in doubt about a water sample, please call the HETL at 287-1716 or the Division of Health Engineering at 287-2070. We will be glad to answer your questions.

Summer is coming and it is time for systems to test for lead and copper again. Within the next few weeks the HETL will be shipping the TE4 kits for lead and copper testing. Schools who receive these kits should sample and return the kits by the end of the school year or by June 30, 1999. Each TE4 sample kit will consist of a quart cubitainer sample bottle, a purple sample information form with specific sampling instructions, and a 141 information form. It is extremely important that the sampling instructions be followed for proper sampling of the system to take place. All forms must be completed and returned with the samples. Failure to return all the forms with the samples may result in the system not receiving credit for the testing and require repeating the testing the next year. Most schools will be receiving five kits. Larger systems could receive ten or twenty kits, depending on the size of the system and the round of testing they are in. If you have questions on the testing, please call the lab at 287-1716 or Health Engineering at 287-2070 and ask to speak with Dana Ivers, Lead and Copper Rule Coordinator.

Lab Certification and Improvement Program

by: Mike Sodano

Maine Medical Laboratory Rule changes.

Effective in early June, revisions to the rules for licensing independent medical laboratories will allow a special license for laboratories that perform only CLIA defined waived tests. The main change that allows this is relaxing the qualification requirements for the General Supervisor (to be similar to Health Screening Laboratory Supervisor requirements). Also the minimum fee for initial (new Laboratory) licenses will increase from $200 to $500.

A one day QA/QC training program for physicians office lab personnel will be presented in Bangor on June 3. Sue Grondin of the Div. Lic. & Cert will present the program assisted by Mike Sodano and the National Laboratory Training Network.

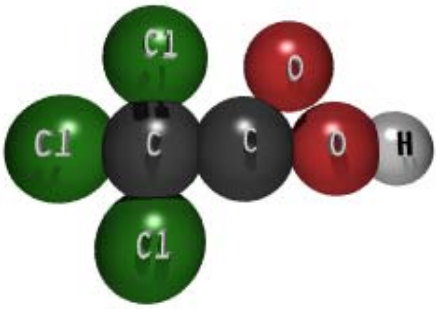
Environmental Certification - Radon in water. The effective date of USEPA regulation of Radon in water is approaching. Currently, the two EPA approved methods are the Lucas Cell method described in the EPA Manual for Certification of Laboratories Analyzing Drinking Water and SM 20th ed. method 7500-Rn. No Maine laboratories are currently certified. Mike Sodano will be working with, Cheryl Baker and Paul Kempf to ensure laboratories are available when compliance monitoring is required.

Disinfection By Products

By: Larry Boston, Organic Section Supervisor

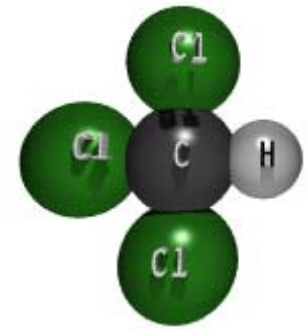
During the disinfection of water there are two major types of compounds that are produced. Both types are now included on the EPA regulated list. The first type of compound is better known to most of you and these compounds have been part of the drinking water testing at HETL for many years. These compounds are the trihalomethanes, which includes chloroform as the major component. The second type of compounds is the haloacetic acids that are higher boiling compounds and require more preparation steps than the trihalomethanes before they can be analyzed. Trichoro acetic acid is usually the major component seen in chlorinated drinking waters. Both of these types of compounds are formed by the action of chlorine on the natural occurring organic material present in the source water.

TRICHLORO ACETIC ACID



The HETL test named TSHAA is for the haloacetic acids. Oxidation of organic materials produce acetic acid, and when the oxidant is chlorine chloroacetic acid compounds are formed. The chlorine used for the purification process usually contains small amounts of bromine, therefore bromine compounds are also produced. There are some bromo-chloro acetic acid compounds which are not regulated at the present time because of difficulties in getting reproducible results for these compounds. The MCL for this set of compounds is based on the average of the total for a four-quarter period. The HETL lab is now sending two sets of results with each sample. The report contains results down to a reporting level established at 5 times the MDL with a < sign before results below the reporting limit. The second report is a spread sheet that contains results reported down to 0. This report makes the reporting of total trihaloacetic acids easier. Any PWSID that received results from the first quarter will receive a revised report reflecting changes in our reporting system.

CHLOROFORM



Another compound produced in the decomposition of organic matter is methane which reacts with commercial chlorine gas to form trihalomethanes. These compounds are included in the TSP test. Chlorine and bromine react with methane to produce trihalomethanes. There are four trihalomethane compounds produced by this process. Chloroform is the primary compound formed as would be expected because bromine is a minor component in the gas mix. The other three compounds usually found in decreasing amounts in the following order dichlorobromomethane, dibromochloromethane and bromoform.

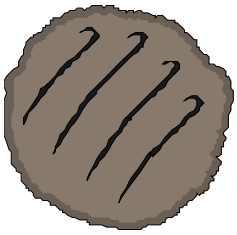
While chlorination remains the major disinfection process the solution to the formation of the disinfection byproducts is to remove the organic matter from the source water. The major process that is now used to remove the organic matter is by passing the water through sand filtration systems. With all of the sand in the world, I was surprised when I visited a water purification system in Connecticut to find out that the best source of sand was obtained from New Jersey.

Note: The halo prefix for the compounds formed by chlorination is derived from the first part of the term halogen (salt formers). The common halogens are Chlorine (**C**L), Bromine (**Br**), Fluorine (**F**) and Iodine (**I**).

Maine's Public Health Laboratory Was A Key Player In A March Ground Beef Recall by Rochester Meats Inc.

By: Donna Wrigley MT (ASCP), B.S.(Mb)

Several suspicious Escherichia coli isolates submitted to the Health and Environmental Testing Laboratory (HETL) during the first three weeks of March were thought to be the cause of a foodborne outbreak occurring at the Sunday River Ski Resort in Bethel, Maine. The isolates serotyped out as O157:H7. The isolates were then DNA "finger printed" using the new Pulsed Field Gel Electrophoresis Laboratory. The patterns from the original three isolates matched completely with each other, indicating a common food source. The DNA pattern was electronically sent to the CDC and ultimately submitted to PulseNet, a national database and computer communication system for reporting DNA patterns of bacteria causing foodborne outbreaks. A total of five isolates, one from a 9 year old from Massachusetts, one from a New Hampshire resident, and three others from Maine, were DNA typed at the HETL. A sixth case, a 6 year old from Massachusetts, was typed at the Massachusetts Institute of Health. Hamburg patties were taken from the restaurant involved and tested by the Federal USDA laboratory. E. coli O157:H7 was found in the hamburger and the DNA electrophoresis pattern matched the pattern submitted to the CDC by Maine's HETL. Massachusetts and Minnesota have both found other isolates matching the outbreak DNA pattern seen in Maine have conducted an epidemiological investigation to determine if these patients were exposed to the same food source.



Rochester Meats Inc., the processing plant involved, recalled 170,780 pounds of product. They supply meat for restaurants and institutions across the country. The recalled products included frozen ground beef patties, ground chuck patties, chopped beefsteak, beef patties, meat for chili, beef patty mix, and pure beef bulk. The meat was produced December 1 and sold in 10-, 15-, and 20- pound packages.

Emerging and Reemerging Infectious Diseases in 1999

By: Tsun-Kong Lee, State Microbiologist

Within the United States, the threat of bioterrorist activity has resulted in the federal government providing more financial grants in developing an expanding role of state public health laboratories. The purpose of this is to develop more advanced biological safety facilities and have rapid response teams to contain and identify biological agents created by people who have hostile intent to the well-being of this country. Even in the absence of such covert threats, the potential danger of exotic infectious diseases entering this country from the African continent through air travelers does exist. The Center for Infectious Diseases has issued updated guidelines on June 30, 1995 for dreaded diseases such as Lassa, Marburg. Ebola and Congo-Crimean hemorrhagic fevers.

In 1995, an outbreak of Ebola in Zaire killed 244 people before it disappeared. In May 1999 when as many as 63 deaths occurred within a mining community in northeastern Congo it was initially believed that Ebola fever had re-emerged. However, it was quickly discovered that the virus was Marburg hemorrhagic fever. This virus was first recognized in 1967 when laboratory personnel were infected in Marburg and Frankfurt, Germany and in Belgrade, Yugoslavia while working with African green monkeys or their tissues in research or polio vaccine preparation. A total of 37 people became ill and the case-fatality was about 25%. Since then a few cases were documented in 1980 and 1987. As with Ebola virus, the actual animal host for Marburg virus is unknown.

The threat of exotic diseases coming only from the African continent has been negated by recent outbreaks of new virus diseases coming from the South Pacific area.. In 1994 a new virus known as Hendra virus infected and killed a small number of horses and humans in Hendra, Queensland, Australia. This virus is a paramyxovirus whose natural host is believed to be fruit bats found in Australia and New Guinea.

Between September 1998 and April 1999, 257 cases of febrile encephalitis with 100 deaths were reported in Malaysia, while in Singapore about a dozen cases were reported. Initially, these cases were believed to be due to Japanese encephalitis virus but since most male patients had close contact with swine another agent was eventually implicated. This new paramyxovirus (formerly known as Hendra-like virus) was called Nipah virus. Because the apparent source of infection among humans was exposure to pigs, approximately 890,000 pigs were killed to control the outbreak in humans.

Finally, a potential threat which has always existed within this country is the presence of four hantaviruses which exist in wild rodents such as deer mice (throughout the United States), white -footed mice (on the Eastern Seaboard) and cotton rats (in the Southeast). The route of airborne transmission from urine and feces can present a problem to people who must disinfect and clean rodent-infested living quarters. The few known deaths documented in the eastern United States suggests that perhaps this disease is not easily acquired since people who work with rodents rarely get it. No other mass outbreak has occurred since May 1993 when 50 fatal cases were reported in the Southwest. One possible explanation for the outbreak suggests that the wet weather the year before yielded bumper yields of seeds, nuts, berries and insects resulting in a huge mouse population explosion in the area. Throughout the country a total of 183 confirmed cases of Hantavirus Pulmonary Syndrome has been reported from 29 states as of mid-1998. The Centers for Disease Control and Prevention has issued recommendations for people like campers and hikers who could be in areas inhabited by rodents and suggests that the risk while small may be reduced even further by following some precautions such as airing out unused or abandoned cabins and disinfecting areas containing

rodent urine and feces with bleach prior to cleaning these areas.

In studying the recommendations for handling clinical specimens such as virus-containing blood and other body fluids in suspected viral hemorrhagic fever cases, the use of universal precautions are generally sufficient to prevent infection and case-to-case transmission by aerosol is unlikely. In Africa the Ebola disease was thought to have been associated with the reuse of non-sterile needles and syringes and close contact and exposure to infectious fluids from patients to physicians and nurses who did not have protective gowns, gloves, goggles and masks to prevent infection early in the outbreak when sick patients have symptoms of vomiting, diarrhea, shock and profuse hemorrhage, and these biological fluids need to be disinfected prior to disposal.

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The State of Maine Bureau of Health proposes to enhance the capacity of its Health and Environmental Testing Laboratory to provide training, planning, end education in advance of possible bioterrorist biological attack. To allow for the testing of biologic agents, and reporting of test results to health, public safety and emergency management agencies, and the general public, in the event of such a possible attack. The Laboratory Biologic Capacity Program is one part of a four pronged statewide effort, including Surveillance and Epidemiology Capacity, Preparedness Planning and Readiness Assessment, and Health Alert Network/Training. The integrated program in response to bioterrorism will enhance the state's capacity to deal with other emergency and chronic public health problems.

Besides naturally occurring disease, the possibility that biologic and chemical agents can be used against the civilian population in terrorist attacks must be addressed. Terrorist use of biological or chemical weapons may be unannounced, or involve overt acts that are announced or otherwise immediately recognized. Absent any immediate evidence or notification of an attack by a perpetrator, the first indications of an attack could be an outbreak of some uncommon illness or an abrupt, significant increase in the incidence of commonly observed symptoms. The speed in which the outbreak is detected, analyzed, understood and addressed, will determine the timeliness and effectiveness of the medical and public health response and hence the extent and severity of the impact upon the health and well-being of the affected community. An unannounced release of a highly communicable disease could afflict many hundreds or thousands of individuals over a wide geographic area. In addition to problems associated with the delayed detection of an agent, many of the agents most likely to be used are not commonly seen as clinical or public health threats in the U.S. It would be the role of the HETL to provide laboratory and consultative support to other responsible agencies associated with emergencies resulting from terrorist incidents.

The jurisdiction covered is statewide with support given to other public health laboratories located in other states upon request . The HETL is close enough to the Greater Boston area to provide support. Being a rural state with county government in place provides an opportunity to affect a coordinated state-wide approach to emergency preparedness. An aspect unique to Maine's is the state's proximity to the greater Boston metropolitan area to our south in addition to bordering the Province of Quebec to our northwest and the Province of New Brunswick to our northeast. There are over 70 unguarded crossing sites under the control of the U.S. Border Patrol. With year-round state ferry service to 6 offshore islands; over 250 airports, airstrips and sea plane bases, and 600 miles of international border with Canada, the State of Maine is vulnerable to terrorist insertion.

Maine's Health and Environmental Testing Laboratory (HETL) is unique in many ways. As a lab of 65 scientists and support staff, the lab is responsible for a large range of testing for the entire state of Maine. In addition to its clinical areas, which include: mycology, mycobacteriology, virology, bacteriology, molecular biology and blood lead, the HETL is multi disciplined and performs analyses in many other fields. These fields cover environmental testing of drinking water, wastewater, groundwater, solid wastes, and pesticides for a full spectrum of inorganic and organic analytes; radiation monitoring, and forensic drug testing for the State Public Safety Department and most Local Police Departments (several are experienced in court room testimony presentations). HETL staff are integrated, so the combined talents are available to State and Federal agencies. The lab is Y2K compliant with a fully integrated laboratory information management system that not only manages laboratory samples through a complete life cycle (all data is on-line since 1988), but also permits independent management of purchasing, accounts receivables and personnel. The HETL is certified by HCFA and EPA, and participates in many proficiency programs.

Pulsed Field Gel Electrophoresis Molecular Strain Typing Available for Nosocomial Outbreaks of Methicillin Resistant Staphylococcus aureus

By: Donna Wrigley MT (ASCP), B.S.(Mb)

Strain typing is an invaluable tool for determining relatedness between isolates in a suspected outbreak. Each species of microorganism has an almost limitless number of strains due to normal random mutations that occur in nature. Phenotypic characteristics such as bacterial biotyping, antibiograms, serotyping, and bacteriophage typing has been used to type strains. Unfortunately, these methods are limited by inconsistencies in their discriminating ability, their labor intensity, and their lack of reproducibility. Pulse field gel electrophoresis (PFGE) is a molecular subtyping method based on genotypic characteristics of an organism. The results produced are clearly interpretable, reproducible, and highly discriminatory. Specific DNA fragments obtained from the whole DNA of an individual isolate are separated by a unique electrophoresis technique capable of separating large DNA fragments, $\geq$ 1000 Kb, as well as small fragments. The DNA "*fingerprint*" produced can then be compared to that of other isolates in order to determine their probable relatedness.

There are three primary applications for strain typing:

1. Assessing relatedness among multiple isolates from an individual patient can help differentiate the true cause of an acute infection from independent contaminants.
2. Differentiate a relapse of an old infection from a new infection on a given patient.
3. Distinguish a true outbreak of a single strain of organism and linking the outbreak to a given source. Identification of the reservoir and mode of transmission of a pathogen is a critical part of recognizing and controlling disease outbreaks, nosocomial or the public community. Differentiating a true outbreak from random coincidental occurrences prevents costly, time-consuming investigations.

We now offer this technology to assist hospital infection control programs and to aid in diagnostic patient care. Typing of Staphylococcus aureus isolates, particularly methicillin resistant strains, is available at **no charge** and we hope to offer PFGE typing for other nosocomial organisms as well.

Maine is currently an active participant of PulseNet. PulseNet is a national network of public health laboratories that performs DNA "fingerprinting" on foodborne bacteria. The network permits rapid comparison of these "fingerprint" patterns through an electronic database at the Centers for Disease Control and Prevention (CDC). The foodborne bacterial pathogens currently being DNA subtyped by the HETL are E. coli O157:H7, Salmonella typhimurium, and other Salmonella species. Other foodborne pathogens for which we have the capacity to type by PFGE are Listeria and Yersinia.

For more information on submitting specimens for molecular subtyping by PFGE please contact the Pulsed Field Laboratory at the following address, fax, or telephone number.

Health and Environmental Testing Laboratory
Donna Wrigley, Microbiologist
PFGE Laboratory
221 State St., Station 12
Augusta, ME 04330

Tel.: (207) 287-2727
FAX: (207) 287-6832

The Great TB Impostor-A Case Study

by: Nancy D. Farrin, Microbiologist

A 42 year old white male presented to his family physician with a six week history of fatigue, cough, intermittent night sweats, and general malaise. The patient was a smoker. His occupation as a long-haul trucker took him all over the United States but primarily to the Mid-West. The patient was treated for a sinus infection with Amoxocillin. One month later, the patient returned to his physician complaining of further malaise, cough, and continuing night sweats. A chest x-ray revealed bilateral upper lobe field density with a left upper lobe cavity. The patient was referred to a specialist for consultation. Tuberculosis was suspected Left and right bronchial washings were performed and sent to the HETL for mycobacterial smear and culture. A PPD was performed. The direct smears of the bronchial washings were negative for acid-fast bacilli and the PPD was negative. Due to the patient's history of severe dental caries, an anaerobic bacterial infection was suspected and he was treated with Penicillin and Flagyl. In two months, the patient returned to the specialist due to severely declining health. He was complaining of weight loss, night sweats, worsening cough, and now a fever. X-rays showed cavitary infiltrates. Clinically, the patient had the classic symptoms of

tuberculosis. A lung biopsy was performed and a sputum sample obtained. Both were sent to the HETL for mycobacterial smear and culture. The lung biopsy was smear negative for acid-fast bacilli. The sputum was smear positive for many, long beaded acid-fast bacilli. The M. tuberculosis direct test (MTD) was ordered and the patient was started on a regimen of TB drugs which included INH, Rifampin, and Ethambutol.

Laboratory Findings

The MTD test was negative for M. tuberculosis. After several weeks incubation the Bactec 12B vials tested positive for growth with an acid-fast bacilli from the lung biopsy and sputum specimens. The Bactec 12B vials from the bronchial washings were positive for acid-fast bacilli The GenProbe assays for M. tuberculosis complex, M. avium complex, and

M. gordonae were completed on all the Bactec broth cultures. All tested negative. The Bactec vials were subcultured to solid media for growth and identification. Approximately three weeks later, the solid media was positive for rough, buff colored colonies. Standard biochemicals were started. The colonies were exposed to light and changed from buff to a strong yellow. The isolate tested strongly positive for catalase, Tween positive in 3 days, positive pyrazinamidase, with a weak to negative nitrate. Growth studies were performed. The isolate demonstrated an intermediate growth rate of 11-14 days. M. kansasii was suspected, but typically is a strong nitrate producer. All four culture isolates tested positive on the GenProbe assay for M. kansasii. The nitrate test was repeated using fresh growth from young cultures and yielded strong positives. The GenProbe assay for M. kansasii was not initially performed due to the low incidence of the organism in Maine. The physician was notified of the identification of M. kansasii. Drug susceptibilities on the isolate were completed by National Jewish in Denver, Colorado.

Conclusion

In culture, M. kansasii has a buff, rough or smooth colony with elevated centers. It is a strong photochromogen. It has an intermediate growth rate of 10-14 days at 37 °C. Key biochemical features are strong nitrate and catalase tests, ability to hydrolyze Tween in 3 days, and positive pyrazinamidase test.

M. kansasii accounts for 3-3.5% of pathogenic mycobacteria isolates in the United States. Geographically, M. kansasii is found in an inverted T pattern across Texas and up into the Mid-West regions of the United States. Geographic clusters of M. kansasii occasionally occur in other areas of the U.S. Trends show M. kansasii infections strike primarily white males who have a predisposing lung condition and/or are smokers.

Pulmonary disease caused by M. kansasii mimics the classic symptoms found with infections of M. tuberculosis. Typically there is upper lobe involvement with evidence of cavities and scarring. Ninety percent of patients with pulmonary disease caused by M. kansasii demonstrate cavitary infiltrates. Extrapulmonary infections are uncommon, although disseminated disease has occurred in patients with AIDS. M. kansasii differs from M. tuberculosis in that it is less virulent and has a slower disease progression. It is believed that M. kansasii infections are environmentally acquired and are not transmitted from person to person.

The current recommendations by the American Thoracic Society for the treatment of pulmonary disease caused M. kansasii is a regimen of INH (300 mg), Rifampin (600 mg), and Ethambutol (25 mg/kg for 2 months then 15 mg/kg) given daily for 18 months.

Presently, the case patient is making slow progress. He has cavitary disease in the left lung with significant scarring. He has gained weight. He has a chest tube in place where the biopsy was performed. He continues to tolerate the drug regimen.

Visual Immunoprecipitate Assay (VIP) for *Listeria monocytogenes* and Related *Listeria* Species Detection in Foods: Comparative Study to Cultural Methods

By: Lisa D. Colson, M.S.

Introduction:

A comparative study on *Listeria sp.* isolation methods from foods has favored a rapid method over the standard Bacteriological Analytical Manual (BAM) cultural method. Five packages of recalled hotdogs were obtained. *Listeria monocytogenes* laboratory stock culture was used as a positive control and uninoculated media was used as the negative control. Twenty-five grams of food sample from one of the 5 hotdog packages tested presumptive positive on the BioControl Visual Immunoprecitate Assay (VIP) and negative on the BAM method. Food samples seeded with positive control culture at varying inoculation levels were simultaneously analyzed by both methods. VIP rapid assay showed superior performance at the lowest levels of inoculum. The lowest inoculum level was undetected by the cultural procedure. Suspicious isolates were observed when VIP selective enrichment broths were subcultured to selective agars. All presumptive positive colonies were confirmed biochemically as *Listeria monocytogenes.*

Methods:

A comparative study was conducted to evaluate the Visual Immunopreciitate Assay (VIP)(1) to the Bacteriological Analytical Manual (BAM)(2) cultural method for the detection of *Listeria monocytogenes* and related *Listeria* spp. in foods. The VIP rapid assay uses highly specific antibodies directed against antigens produced by *Listeria*. Liquid sample initiates lateral flow along the solid support surface of a single-use device.

To perform this assay, 25 grams of hotdog was added to 225 mL modified Frases broth and stomached for 2 minutes. The hotdog broth emulsion was incubated for 28±2 hours at 30°C. A 1.0 mL sample of broth was then transferred to 9.0 mL buffered *Listeria* enrichment broth (BLEB). This secondary enrichment was incubated for 24±2 hours at 30°C. A 1.0 mL portion of the secondary enriched broth was inactivated by heating at 100°C for 5 minutes. A 0.1 mL portion of heat inactivated enriched broth was transferred to the sample well on the VIP device. This initiates lateral flow of the broth across the surface of the solid support. *Listeria* in the sample reacts with an antibody-chromagen complex in the device. The antigen-antibody-chromagen complex flows across lateral-flow membrane and binds to antibody immobilized on the membrane, forming a detection line in a viewing window. A second line forms in a procedural control window indicating the completion of the test. After 48 hour enrichment, the assay reaction is complete within 10 minutes from the time inoculum was added to the device test well. Reserved enriched broth of presumptive samples were plated on selective plating media and suspicious isolates

characterized biochemically on the Vitek 32 rapid identification system.

The BAM procedure detects *Listeria* in foods by cultural methods. Twenty-five grams of food sample was added to 225 mL Listeria Enrichment Broth , stomached for 2 minutes and incubated at 30 °C for 4 hours. After this resuscitation step, 0.445 mL acriflavin and 1.8mL nalidixic acid were added as selective agents. One selective agent, cyclohexamide was omitted due to harmful health effects. The enrichment broth was incubated at 30 °C for a total of 44 hours. At 24 hours and 44 hours of incubation, 0.1 mL of enrichment broth was plated onto each of Oxford agar and PALCAM agar plates. Plates were incubated at 35°C for 24 - 48 hours, CO_2 atmosphere is optional. Colonies with black halos are typical for *Listeria sp.*. Five colonies from each plate were picked to Triptic Soy Agar with Yeast Extract (TSAYE) plates, incubated at 35°C for 24 hours. Pure cultures were processed for catalase and Gram stained. Rapid identification was performed by the Vitek 32. To differiate *Listeria* species the hemolysis on Blood Agar, and utilization for the following sugars: rhamnose, mannitol, and xylose was also performed. *Listeria monocytogene*s are beta-hemolytic and metabolize only rhamnose.

To test varying inoculation levels of *Listeria monocytogenes*, a stock culture was grown up in Brain Heart Infusion broth. The broth culture was serial diluted in Butterfield's Buffer. Five of the 8 dilutions were used to inoculate separate 25 gram samples of hotdogs previously tested negative for *Listeria sp*. The 5 inoculation levels were analyzed by the VIP rapid assay and BAM culture method.

Results:

Of the 5 packages of hotdogs analyzed, only 1 package was confirmed positive by the VIP method. All 5 packages were negative by the BAM culture method. Samples inoculated with *Listeria monocytogenes* were quantitated by direct plating on selective agar. The 5 inoculated samples , all confirmed positive by the VIP method. The BAM culture method confirmed positive for 4 levels of inoculum, but gave a false negative result at the lowest inoculation level(Table 1).

Table 1. Results of hotdogs analyzed for the presence of *Listeria*

Sample #/Inoculum level	BAM method	VIP method	Confirmed ID
Positive Control	Positive	Positive	Positive
Negative Control	Negative	Negative	Negative
Hotdog Package 1	Negative	Negative	Negative
Hotdog Package 2[a]	**Negative**	**Positive**	**Positive**
Hotdog Package 3	Negative	Negative	Negative
Hotdog Package 4	Negative	Negative	Negative
Hotdog Package 5	Negative	Negative	Negative
$9.7X10^5$ CFU/g	Positive	Positive	Positive
$9.7X10^3$ CFU/g	Positive	Positive	Positive
97 CFU/g	Positive	Positive	Positive
0.97 CFU/g	Positive	Positive	Positive
0.0097 CFU/g[b]	**Negative**	**Positive**	**Positive**

[a] BAM method was negative, VIP was positive, *Listeria* was isolated .

[b] BAM method was negative, *Listeria* recovered from this inoculum.

Discussion:

Of 5 packages of hotdogs, only 1 confirmed positive for *Listeria sp.* by the VIP rapid method and tested negative by BAM cultural method. All VIP selective enrichment broths were subcultured to selective agars confirming the presence of *Listeria sp.*. It was observed that the VIP enrichment protocol for isolating *Listeria sp*. from naturally contaminated hotdogs has better selective properties than that used in cultural methods, allowing the slow growing *Listeria* to resuscitate and propagate (Table 1). The use of different primary broths for culture methods and the VIP method may explain some of the differences between the 2 methods. Other comparative studies have reported similar results(3). The Oxford selective agar consistently showed better growth of *Listeria sp.* than did the PALCAM. The *Listeria monocytogene*s spiking level in this study was quite low by design considering natural contamination levels and that *Listeria* are relatively slow growing microorganisms. Feldsine et al (3) data indicated that the VIP method and the BAM/USDA culture methods were comparable for detection of *Listeria monocytogenes* and related *Listeria sp*. in selected food types.

Conclusion:

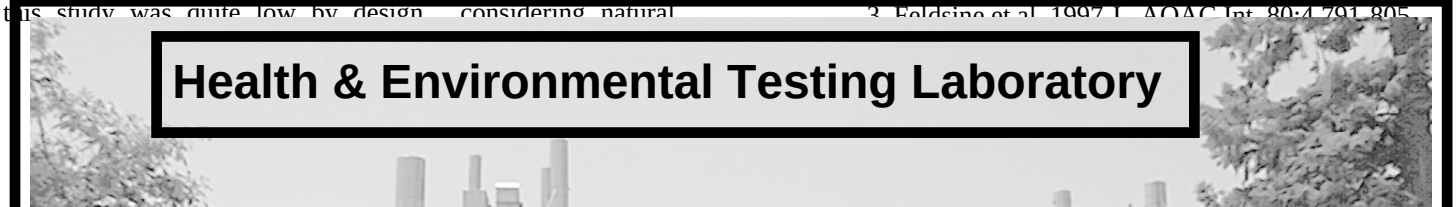
On the basis of this study, it is recommended that the VIP method for detection of *Listeria monocytogenes* and related *Listeria sp.* in foods be adopted as the preferred method for recovery of *Listeria*, followed by colonization on Oxford selective plating agar and confirmed identification by the Vitek 32 system.

References:

1. BioControl Systems, Inc., 1997. AOAC Official Method 997.03 Bellevue, WA 98005.

2. Bacteriological Analytical Manual 1995. 8th Ed., AOAC International, Gaithersburg, MD.

3. Feldsine et al. 1997 J. AOAC Int. 80:4 791-805.



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